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Ottawa Hull K1A 0C9

(11) (C) **1,339,798**

(21) 499,927

(22) 1986/01/20

(45) 1998/04/07

(52) 167-139

(51) Int.Cl.[6] A61K 39/395

(19)(CA) **CANADIAN PATENT** (12)

(54) Method for Treatment of Neuroectodermal Malignancies and Epithelial Carcinomas in Humans

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(30) (US) U.S.A. 697,264 1985/02/01

(57) 8 Claims

NO DRAWING

ABSTRACT

This invention provides a use of monoclonal antibody R-24 linked to a chemotherapeutic agent for the treatment of cancer of neuroectodermal origin, epithelial cancer of pigmentation disorder and wherein the chemotherapeutic agent is at least one agent from the group dacarbazine, adriamycin, pimozide, dibromodulcitol, bleomycin, actinomycin D, purine analog, pyrimidine analog, nitrosoureas, cis-platin, radiation-emitting reagent, tumor necrosis factor, interferon, lymphotoxins, interleukin, BCG, and autologous tumor cell vaccine.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Use of monoclonal antibody R-24 linked to a chemotherapeutic agent for the treatment of cancer of neuroectodermal origin, epithelial cancer or pigmentation disorder, wherein the chemotherapeutic agent is at least one agent from the group dacarbazine, adriamycin, pimozide, dibromulcitol, bleomycin, actinomycin D, purine analog, pyrimidine analog, nitrosoureas, cis-platin, radiation-emitting reagent, tumor necrosis factor, interferon, lymphotoxins, interleukin, BCG (Bacillius Calmette-Guérin), and autologous tumor cell vaccine.

2. The use of claim 1, wherein the epithelial cancer is lung or breast carcinoma.

3. The use of claim 1, wherein the neyroectodermal cancer is selected from the group of cancer consisting of metastic malignant melanoma, astrocytomas and neuroblastomas.

4. The use of claim 1, wherein the nitrosourceas are selected from the group consisting of CCNU (N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea), BCNU (N,N'-Bis(2-chloroethyl)-N-nitrosourea) and methyl CCNU.

5. The use of claim 1, wherein the purine analog is 6-mercaptopurine or 2,6-diaminapurine.

6. The use of claim 1, wherein the pyrimidine analog is 5-fluorouracil.

7. The use of claim 1, wherein the radiation-emitting reagent is ^{60}Co, ^{131}I or ^{90}yttrium.

8. The use of claim 1, wherein the interleukin is interleukin 1 or interleukin 2.

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This invention was supported in part by grants from the National Cancer Institute (CA-19267 and CA-08748). Therefore the United States Government has certain rights in this invention.

Summary

This application concerns treatment of neuroectodermal disorders or cancers, espeically melanoma cancers in humans with mouse monoclonal antibody R_{24}, an IgG_3 antibody to GD_3 cell surface ganglioside restricted to certain cells of neuroectodermal origin.

BACKGROUND

Mouse monoclonal antibodies have defined a large number of antigens on the surface of melanoma cells (Lloyd, K., (1983) R.B. Herberman, ed. IN: Basic and Clinical Tumor Immunology Martinus Nijoff Publ., Boston, M.A., U.S.A.; Reisfeld, R.A., Ferrone, S. (eds) (1982) Melanoma Antigens and Antibodies Plenum Press, New York). Although none of the antigens are melanoma-specific, some antigens have characteristics of differentiation antigens that mark melanocytes, melanoma and other cells of neuroectodermal origin (Houghton, A.N., et al. (1982) J. Exp. Med.

156:1755-1766). An IgG3 mouse monoclonal antibody, designated R_{24}, identifies one of the most restricted of these neuroectodermal markers. R_{24} was generated by Dippold et al. (Dippold, W.G., et al. (1980) Proc. Nat'l. Acad. Sci., U.S.A. 77:6114-6118) during a study of surface antigens of cultured melanoma cells and Pukel et al. (Pukel, C.S., et al. (1982) J. Exp. Med. 155: 1133-1147) (U.S. Patent No. 4,507,391 issued March 26, 1985) demonstrated that R_{24} identified the disialoganglioside GD_3. Analysis of cultured cells (Dippold, W.G., et al. (1980) Supra) and normal and malignant tissues (Real, F.X., et al. (1982) Proc. Am. Assoc. Cancer Res. 23:1006) showed that R_{24} reacts with melanocytes, astrocytes, melanomas, astrocytomas, and a subset of sarcomas. R_{24} also mediates a variety of biological effector functions, including tumor cell aggregation, human complement-mediated cytotoxicity, and antibody-dependent cell-mediated cytotoxicity with human complement-mediated cytotoxicity, and antibody-dependent cell-mediated cytotoxicity with human effector cells (Dippold, W.G. et al. Cancer Res. 44:806-810; Knuth, A., et al. (1984) Proc. Am. Assoc. Cancer Res. 25:1005; Vogel, C-W, et al. (1983) Immunobiol. 164:309). R_{24} is on deposit at the American Type Culture Collection, 1230 Parklawn Drive, Rockville, MD. 20852 since November 29, 1983 and has the accession # HB8445.

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Patients' tumors were shown to express G_{D3} by indirect immunofluorescence tests on frozen sections prior to treatment. All patients had objectively measureable disease, a performance status (Karnofsky scale) of at least 60, and were off anticancer therapy for at least four weeks. No concurrent anticancer therapy was given during evaluation. Patients were considered evaluable six weeks after initiation of therapy; ten patients were evaluable and two patients had not yet reached the six week mark. The phase I trial with R_{24} antibody was approved by the Institutional Review Board of Memorial Hospital; informed consent was obtained from all patients.

DESCRIPTION

The examples shown serve to illustrate the invention without limiting it.

In the present study, we show the response of melanoma patients to R_{24} with regard to different dose levels, toxicity, serological parameters and tumor response.

Preparation and Administration of R_{24}

R_{24} was prepared from ascites of (BALB/C x 57 Bl)F_1 mice, purified by ammonium sulfate precipitation, chromatography over protein A Sepharose* with pH 4.0 acetate buffer elution and further purification by G-25 Sephadex* column with PBS pH 6.0 and filtered. Each R_{24} batch was 3 mg/ml stored in 2% human serum albumin at -70°C. Each batch was tested for antibody reactivity and assayed for nucleic acids, 16 mouse viruses, bacteria, fungi and mycoplasma. Preparations underwent standard safety testing in mice and guinea pigs and pyrogenicity testing in rabbits.

R_{24} was administered by intravenous infusion in 100-200 ml 0.9% saline and 5% human serum albumin. Skin tests with 0.1 micro g R_{24} were done before the first treatment. The schedule of treatment was 1 mg/M^2 or 10 mg/M^2 every other day for eight treatments or 30 mg/M^2 per day by continuous in fusion on days 1 through 5 and 8 through 12.

Serological Tests

R_{24} antibody titers were determined by testing serum samples in protein A mixed adsorption assays (PA assays) (Pfreundschuh, M., et al. (1978) Proc. Nat'l. Acad. Sci., U.S.A. 75:5122-5126) against the melanoma target cell

*Trademark

line SK-MEL-28. R_{24} concentrations were measured by an enzyme-linked immunoassay. Falcon* 3034 plates (Falcon Labware, Oxnard, CA, U.S.A) were precoated with purified R_{24} 125 micrograms/ml. Rabbit antimouse IgG3 (Bionetics, Inc., Kensington, MD U.S.A.) diluted 1:100 was mixed (1:1 vol/vol) with patients' serum samples diluted 1:4 and incubated for 120 min. The mixture was transferred to the precoated wells, incubated for 60 min, and wells were washed with phosphate-buffered saline (PBS). Wells were incubated with goat antirabbit IgG linked to alkaline phosphatase (Sigma Chemical Co., St. Louse, MO, U.S.A.), for 60 min. Alkaline phosphatase activity was determined using p-nitrophenyldisodium phosphate substrate (Houghton, A.N., et al. (1983) J. Exp. Med. 158:58-65. R_{24} concentrations were determined by comparison to standards using different concentrations of purified R_{24} diluted in a pretreatment serum sample from the patient.

Human IgG antibody against mouse Ig was detected by enzyme-linked immunoassays. Falcon 3034 plates precoated with R_{24} 50 micrograms/ml were incubated with patients' serum samples diluted 1:50 for 60 min, and washed with PBS. Antihuman IgG linked to alkaline phosphatase (Sigma Chemical Co.), was incubated in wells for 60 min, and reaction were measured by spectrophotometry (Houghton, A.N., et al. (1983) Supra).

*Trademark

Indirect immunofluorescence and immunoperoxidase procedures were performed as previously described in the art (Erlandson, R.A., et al. (1984) Am. J. Surg. Path. (in press)). The following reagents (Ortho Diagnostic Systems, Raritan, NJ, U.S.A.) were used for testing tissue sections: OKT-3, OKT-4, OKT-8 antibodies to T cell markers; OKB-2 and OKB-7 antibodies to B cell markers; OKM-1 and OKM-5 antibodies to macrophage markers; and OKIa-1 to human Ia antigens. Goat antisera to the human complement components C3, C5 and C9 were provided by Dr. Carl-Wilhelm Vogel. R_{24} was used at concentration of 40 micrograms/ml. Mouse IgG in tumor tissues was detected by incubating section directly with biotinylated antimouse IgG, then with avidin-peroxidase conjugates and substrate. The toluidine blue staining method was used to detect tissue mast cells.

The examples below are for illustrative purposes and are not meant to limit the invention.

Patient Characteristics

Table I lists the clinical features of the 12 patients included in the study. The patients received total doses of 8 mg/m^2 (three patients), 80 mg/m^2 (six patients) and 240 mg/M^2 (three patients). The median age was 40 years (range 25-67) and the median performance status was 70

(range 60-90). Six patients had received prior chemotherapy, radiation therapy or interferon treatment. All patients had skin or soft tissue disease. In addition, visceral metastases were present in seven patients, including lung (three patients), brain (three patients) and liver (one patient).

Toxicity

No side-effects were observed in the three patients treated with the lowest does of R_{24} (8 mg/M^2). All patients receiving a total dose of 80 mg/M^2 or greater had skin reactions, manifested by urticaria and pruritus usually developing 2-4 hrs after starting treatment. The intensity of skin reactions was related to the dose level and rate of antibody infusion. Urticaria characteristically appeared over tumor sites in the skin and subcutaneous soft tissue and around surgical scars where tumor had been removed. One patient (patient 6) developed urticarial lesions at sites where she had received melanoma cell vaccines/(ref) eight months previously. No reactions developed at R_{24} skin test sites or around surgical scars unrelated to tumor treatment. Six patients went on to develop more generalized urticarial

lesions over the face, trunk or limbs (patients 5,7,8,10,11,12). Patient 5 experienced mild wheezing after rapid infusion of antibody (10 mg/hr); in this case the dose of R_{24} was reduced to 66% of the total intended dose. Diphenhydramine was effective in controlling side-effects, but was only used for systemic symptoms.

At a dose level of 80 mg/M, the severity of skin reactions, particularly pruritus was found to be related to the rate of infusion of R_{24}. Treatment was tolerated well when the infusion rate was maintained at less than 5 mg/hr. At this rate, skin reactions usually occurred only after the first, second and third infusions and not after subsequent treatments. AT 240 mg/M^2, R_{24} was administered by continuous infusion to maintain an infusion rate below 5 mg/hr. All three patients treated at this dose level developed urticaria initially restricted to tumor sites which later became generalized. Patients 10 and 11 experienced mild nausea and vomitting between 4 and 8 hrs after the start of treatment. Temperature elevation (up to 37.8°C) was seen in patients 11 and 12 near the end of treatment. No hepatic, renal, hematopoietic or neurological toxicity was observed and no changes were noted in vision or skin pigmentation over a period of up to 9 months follow-up.

Antitumor Effects

Table I summarizes tumor responses in patients treated with R_{24}. Major tumor regression was observed as illustrated.

Example 1

Patient 3, is a 36-year-old woman with primary malignant melanoma of the back, Clark's Level IV, 5mm depth of invasion diagnosed 9/82. In 8/83, the patient was found to have recurrent tumor and on 11/30/83, treatment with R_{24} was started, 1 mg/M^2 (1.7 mg) every other day for eight doses. No toxicity or reactions at tumor sites were noticed during treatment. Sites of measurable disease included a firm 7 x 9 cm right axillary mass, a 4 x 2.5 cm subcutaneous nodule over the right hip and a 3 x 4.5 cm right paratracheal mass. A poorly defined density was present in the right upper lobe of the lung. Regression of tumor in the axilla and paratracheal region was first observed five weeks after starting treatment. The paratracheal mass has been undetectable since 3/84. The right axillary mass measured 1.2 x 0.8 cm in 5/84 and has continued to regress. The subcutaneous nodule over the right hip did not change in size but became very tender and inflamed by 2/5/84. Excisional biopsy of this lesion revealed hemorrhagic necrosis and inflammatory cell infiltrates with small nests

of melanoma cells which stained weakly or not at all with R_{24} By 5/84, the density in the right upper lung field had become better defined, and a needle biopsy revealed melanoma cells which reacted strongly with R_{24}.

Example 2

Patient 4 had a malignant melanoma diagnosed 2/82, 7 mm thickness, Clark's Level IV, with tumor in 10 of 12 regional lymph nodes. By 1/84, the patient had extensive bulky skin and soft tissue metastases (over 100 lesions) on the left thigh, in the left and right inguinal areas, and on the lower abdomen, scrotum and penis. Treatment with R_{24}, 20 mg/M^2 every other day for eight doses, was started on 1/18/84. Two hours after the start of the first infusion, the patient developed severe pruritus and urticarial lesions around all tumor sites. Urticaria progressed to confluent erythrema over tumor sites and adjacent areas of the thigh, inguinal areas and lower abdominal wall. These reactions disappeared 18 hrs later. A milder reaction was seen after the second and third doses and no reactions were seen during subsequent treatments. Eight weeks following the end of R_{24} treatment, there was enlargement of lesions in the right groin and left thigh and new lesions had appeared over the abdominal wall. However, four weeks later, all measurable lesions had decreased in size by greater than 50%. There

has been continued tumor regression over the past six months and most sites are now tumor-free.

Example 3

Patient 10 is a 61-year-old man who developed a melanoma, 2.8 mm thickness, Clark's Level III, over the right scapular area in 1977. A solitary lesion of the left frontal lobe of the brain was detected in 1/82 and treated by left frontal lobe craniotomy followed by whole brain radiation therapy. Between 6/83 and 10/83, multiple subcutaneous tumors developed over the trunk, and the patient continued to progress during sequential treatment with dacarbazine, Pimozide, CCNU, and dibromodulcitol. Treatment with R_{24}, 240 mg/M^2, by intravenous infusion over two weeks was started on 6/11/84. The patient had more than 30 skin and soft tissue lesions on the trunk, extremities, scalp, face and neck measuring between 1 and 5 cm in diameter. Four hours after starting treatment the patient developed urticaria first around tumor lesions and then becoming generalized by six hours. The skin reactions abated over the next 12 hours and were gone by the fourth day of treatment. Regression (greater than 90%) of several pigmented tumors was seen at the end of R_{24} treatment, and generalized regression of lesions was observed by four weeks

after starting therapy. During tumor regression, subcutaenous ecchymoses were noted over six responding tumor sites. The patient achieved a partial remission (greater than 50% regression of measurable lesions) and regression of lesions has continued. In the face of regression of skin and soft tissue lesions, the patient developed left mild hemiplegia eight weeks after the start of therapy. CTT image of the brain demonstrated an enlarging lesion in the putamen with a necrotic center.

Example 4

Mixed responses were observed in two other patients (patients 2 and 9) (Table I). Patient 7 and rapid progression of bulky skin and soft tissue disease with deterioration of performance status and received dacarbazine five weeks after the start of R_{24} treatment; she achieved a partial response three weeks after dacarbazine treatment and has remained in remission for more than 20 weeks. Patient 5 also showed progression of disease with development of new skin nodules at six weeks. Treatment with dacarbazine resulted in a partial response lasting now more than 20 weeks.

Serology

Table II presents serological studies performed on patients treated with R_{24} which show:

1. There was heterogeneity in the expression of G_{D3} in the melanoma specimens obtained before treatment, ranging from 40% positive cells to 100% positive cells.

2. Peak R_{24} levels were related to the amount of antibody received. Median peak R_{24} levels were 0.8 microgram/ml at 8 mg/M^2, 7 microgram/ml at 80 mg/M^2, and 58 microgram/ml at 240 mg/M^2.

R_{24} levels fell off rapidly after the last R_{24} treatment and were usually less than 5% of peak levels by 18 hrs after the end of therapy.

3. Elevated levels of human IgG against mouse Ig were detected in all evaluable patients between 15 and 40 days after the start of therapy.

4. There was no evidence of antigenic modulation during therapy. Tumor cells biopsied or aspirated during therapy in patients 4 and 10 demonstrated continued expression of G_{D3} in the face of substantial levels of circulating and tumor-bound R_{24}.

5. Progression of tumor after R_{24} treatment was not related to outgrowth of G_{D3} negative cells. Biopsies done after tumor persistence of progression showed strongly G_{D3} positive melanoma cells in the lung lesion of patient 3 and skin lesions of patients 5, 6 and 9.

6. The amount of R_{24} reaching tumor cells appeared to correspond to the dose level of antibody given. Mouse IgG was not detected in lesions from a patient treated at 8 mg/M^2 (patient 2), but was detected weakly around vessels in patient 4 (80 mg/M^2) and strongly around vessels and on a proportion of tumor cells in patient 10 (240 mg/M^2).

7. Inflammation at tumor sites involved several components associated with immune reactions. Tumors from patients 4 and 10 had increased numbers of mast cells with evidence of mast cell degranulation, evidence of complement deposition including C3, C5 and C9, and infiltration with $T8^+/Ia^+$ lymphocytes. Tumor tissue taken immediately before treatment did not demonstrate these charcteristics.

A variety of mechanisms may be involved in the antitumor activity of R_{24}, ranging from antibody-directed complement-mediated cytotoxicity and cell-mediated

cytotoxicity, to tumor cell injury secondary to the inflammatory reaction elicited in the tumor bed by R_{24}. Tumor biopsies showed a range of inflammatory changes in R_{24}-treated patients, with infiltration of T cells and mast cells, mast cell degranulation, and deposition of C3, C5 and C9 complement components being among the most prominent. Dippold et al. have also observed inflammatory reactions over tumor sites in two patients treated with R_{24} [Dippold, W.G., et al. Proc. Am. Assoc. Cancer Res. 25:978 (abstract]). However, no tumor regression was found.

In our studies of a large number of mouse monoclonal antibodies to surface antigens of human cancer, R_{24} has been found to be unique in its ability to activate human complement to such a high degree and to induce extremely strong cell-mediated cytotoxicity. Whether these are general characteristics of antibodies (like R_{24}) that belong to the IgG3 subclass or are related to the nature of the G antigenic determinant is not known. These questions can be addressed by studying other classes and subclasses of G_{D3} monoclonal antibodies and IgG3 antibodies to either surface antigens. Complement components, such as C5a, that are generated during complement activation are known to have inflammatogenic activity (Morgan, E.L., et al. 91984) Fed. Proc. 43:2543-2547.), and it is likely that these are

involved in R_{24}-directed inflammation at the tumor site. If complement activation plays a key role in R_{24}-induced inflammation and antitumor effects, attention needs to be directed at complement levels, both systemic and intratumoral, during R_{24} therapy to determine whether complement availability might limit the therapeutic activity of R_{24}.

Another aspect of R_{24} that needs clinical evaluation is the possibility that responses to chemotherapy may be enhanced or potentiated by R_{24}. Studies in nu/nu rats and mice have shown that the antitumor effect of R_{24} is potentiated by drugs such as adriamycin. Increased tumor blood flow and altered susceptibility of tumor cells to the action of drugs as a consequence of antibody treatment are two possibilities that could account for increased drug sensitivity of R_{24}-treated animals. This observation may have its counterpart in the clear responses of two R_{24}-treated patients to dacarbazine. Thus R_{24} could be combined with other chemotherapeutic anti-tumor agents selected but not limited to those such as the purine or pyrimidine analogues, the nitrosoureas, cisretinoic acid, pimozide, dibromodulcitol, DTIC, cytoxan, methotrexate hydroxyurea, 6-mercapto-purine, adriamycin, cisplatin, bleomycin, actinomycin D and/or radiological treatment such

as ^{60}Co or ^{90}yttrium, ^{131}I or ^{211}astatine. Natural agents or factors such as tumor necrosis factor (TNF) [Williamson et al (1983) Proc. Nat'l. Acad. Sci. U.S.A. 80:5397], BCG, autologous tumor cell vaccine [Houghton et al in Immunodermatology ed. by Bigan Safai and Robert A. Good Plenum, 1981, see p.570], Interferon, Interleukin-2 (See Welte et al (1982) J. Exp. Med. 156:454), Interleukin-1 and Lymphotoxins in general can also be used in conjunction with R_{24}, administered together or in linkage form. Some nitrosourea for example are BCNU (1,3-BIS(2-chloroethyl)-1-nitrosourea], CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and methyl CCNU. Purine and pyrimidine analogues may include 6-mercaptopurine, 5-fluorouracil, and 2,6 diaminapurine for example. Others will be apparent to those skilled in the art.

Also available for clinical use will be a link of the mouse variable region of R_{24} to a human F_c portion for a more human-like immunoglobulin. This can be done by linking the DNA region coding for the mouse variable portion of the Ig molecules with a DNA region coding for the F_c portion of human immunoglobulin to produce a mixed R_{24} with a human F_c component which may be tolerated better at higher doses. It is also possible to link R_{24} molecules to fluorescent agents to localize tumors.

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Also these could be linked to R_{24} to localize as well as treat the tumor in situ. R_{24} has already been linked to ^{131}I to localize as well as treat tumors such as melanomas. Melanocyte disorders such as Nevi or pigmentation disorders could be treated as well. R_{24} has also been found useful in treatment since it reacts with epithelial carcinomas such as those of lung and breast. The listing above is meant as a series of examples and is by no means limiting to the invention as described. Other examples will be apparent to those skilled in the art.

However, a dose-response relation to R_{24} may not be straightforward, since patients' responses will be influenced by a number of other parameters, e.g., strength and heterogeneity of G_{D3} antigen expression, tumor cell accessibility, and availability of accessory factors such as complement, histamine and inflammatory cells.

TABLE I

CLINICAL FEATURES AND TUMOR RESPONSES OF PATIENTS TREATED WITH R_{24} MOUSE MONOCLONAL ANTIBODY

	AGE	SEX	PRIMARY SITE	PERFORMANCE STATUS (KARNOFSKY SCALE)	PRIOR THERAPY	DOSE LEVEL (TOTAL DOSE)	SITES OF DISEASE	RESPONSE AND DURATION (BY SITE)
1	55	F	Foot	90	Intralesional	8mg/M^2 (16.8 mg)	Skin*	Progression after 4 weeks
2	57	F	Leg	70	Radiation therapy	8mg/M^2 (13.6 mg)	Skin*	Partial response[1], 10 weeks
							Brain	Stable, 19 weeks
3	36	F	Trunk	90	BCG, Cisretinoic acid	8mg/M^2 (13.6 mg)	Axilla and Paratracheal Lymp Node*	Partial response[1], 38+ weeks
							Lung	Progression after 24 weeks
4	30	M	Leg	70	Interferon, Intralesional therapy	80mg/M^2 (168 mg)	Skin and Lymph Nodes*	Partial response[1], 28+ weeks
5	52	M	Unknown	70	Intralesional therapy	80mg/M^2 (90 mg)	Skin and Lymph Nodes*	Progression after 6 weeks[2]
6	25	F	Neck	70	Autologous tumor cell vaccine	80 mg/M^2 (144 mg)	Skin*	Progression after 10 weeks
							Brain	
7	44	F	Leg	70	Intralesional therapy	80 mg/M^2 (136 mg)	Skin and Nodes (axilla, retroperitoneum)*	Progression after 5 weeks[2]
8	30	M	Choroid	60	Methotrexate, BCNU, DTIC, Hydroxyurea	80mg/M^2 (144 mg)	Skin*	Progression died at 6 weeks
							Liver*	

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TABLE I Cont'd.

CLINICAL FEATURES AND TUMOR RESPONSES OF PATIENTS TREATED WITH R_{24} MOUSE MONOCLONAL ANTIBODY

	AGE	SEX	PRIMARY SITE	PERFORMANCE STATUS (KARNOFSKY SCALE)	PRIOR THERAPY	DOSE LEVEL (TOTAL DOSE)	SITES OF DISEASE	RESPONSE AND DURATION (BY SITE)
9	67	M	Trunk	90	DTIC, Interferon	80mg/M^2 (152 mg)	Skin*	Progression after 6 weeks
							Lung*	Partial response[1], 10 weeks
10	58	M	Trunk	70	DTIC, CCNU, Dibromodulcitol, Pimozide	240 mg/M^2 (528 mg)	Skin*	Partial response[1], 6^+ weeks
11	34	F	Leg	90	DTIC, Actinomycin D	240mg/M^2 (400 mg)	Retroperitoneal Lymph Nodes*	Stable, 4^+ weeks
12	26	F	Leg	90	Cytoxan	240mg/M^2 (400 mg)	Skin*	Stable, 4^+ weeks
							Mediastinum*	Stable, 4^+ weeks
							Lung*	Stable, 4^+ weeks

*Sites of measurable disease.

[1]Partial response - greater than or equal to 50 reduction in the sum of the products of the maximum and perpendicular diameters of all measurable lesions for at least 4 weeks.

[2]Patients 5 and 7 subsequently achieved partial responses with dacarbazine treatment.

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TABLE II

SEROLOGY AND IMMUNOPATHOLOGY OF PATIENTS TREATED WITH R_{24}

PATIENT	R_{24} REACTIVITY WITH MELANOMA BIOPSIES (PRETREATMENT)		R_{24} SERUM LEVEL					HUMAN IgG ANTI-MOUSE Ig POST/PRETREATMENT[3] (DAYS AFTER START OF TREATMENT)	IMMUNOPATHOLOGY (DAYS AFTER START OF TREATMENT)
			PEAK			18 HRS FOLLOWING THE END OF TREATMENT			
	INTENSITY (0-3+)	% + Cells	Day	Titer[1]	Mouse IgG_3[2]	Titer	Mouse IgG_3		
1	3+	100%	4	1/64	0.8 ug/ml	1/8	L.T. 0.1 ug/ml	4.1 (164 days)	Not examined
2	3+	80%	7	1/32	L.T. 0.1 ug/ml	0	L.T. 0.1 ug/ml	5.6 (70 days)	Increased infiltration with lymphocytes and mast cells. Frequent mast cell degranulation. No mouse IgG detected. (Day 13)
3	2+	70%	9	1/128	2 ug/ml	0	L.T. 0.1 ug/ml	3.9 (100 days)	Increased infiltration with lymphocytes and mast cells. (Day 64)
4.	3+	100%	7	1/256	13 ug/ml	1/32	0.5 ug/ml	3.8 (80 days)	Increased infiltration with $T8^+/Ia^+/T3^+/T4^-/B2^-/B7^-/M1^-/M5^-$ lymphocytes. Increase in the number of mast cells with frequent degranulation. Deposition of complement (C3,C5,C9). Mouse IgG in tumor biopsy primarily around vessels. (Day 13)
5.	2+	100%	7	1/256	3 ug/ml	1/16	L.T. 0.1 ug/ml	2.1 (40 days)	Increased infiltration with lymphocytes and mast cells. Frequent mast cell degranulation. (Day 32).
6.	2+	70%	7	1/128	7 ug/ml	1/32	L.T. 0.1 ug/ml	2.0 (40 days)	No inflammatory infiltrate. (Day 64).
7.	2+	80%	14	1/512	5 ug/ml	1/128	1 ug/ml	2.3 (40 days)	Not examined.

TABLE II Cont'd.

SEROLOGY AND IMMUNOPATHOLOGY OF PATIENTS TREATED WITH R_{24}

PATIENT	R_{24} REACTIVITY WITH MELANOMA BIOPSIES (PRETREATMENT)		R_{24} SERUM LEVEL					HUMAN IgG ANTI-MOUSE Ig POST/PRETREATMENT[3] (DAYS AFTER START OF TREATMENT)	IMMUNOPATHOLOGY (DAYS AFTER START OF TREATMENT)
			PEAK			18 HRS FOLLOWING THE END OF TREATMENT			
	INTENSITY (0-3+)	% + Cells	Day	Titer[1]	Mouse IgG_3[2]	Titer	Mouse IgG_3		
8.	2+	80%	4	1/64	6 ug/ml	0	L.T. 0.1 ug/ml	1.3 (35 days)	Not examined.
9.	2+	70%	9	1/256	8 ug/ml	0	L.T. 0.1 ug/ml	3.3 (40 days)	Not examined.
10.	3+	100%	12	1/1024	58 ug/ml	1/128	(N.T.)	1.8 (30 days)	Infiltration with $T8^+/Ia^+/T3^+/T4^-/B2^-/B7^-/M1^-/M5^-$ lymphocytes. Increase in the number of mast cells with frequent degranulation. Deposition of complement (C3,C5,C9). Mouse IgG in tumor biopsy around vessels and in tumor. (Day 11)
11.	3+	90%	5	1/256	18 ug/ml	1/128	1 ug/ml	1.2 (20 days)	Not examined.
12.	2+	40%	5	1/800	62 ug/ml	1/200	2 ug/ml	1.3 (14 days)	Not examined.

[1] R_{24} serum titers measured by PA mixed hemadsorption assay against SK-MEL-28 melanoma target cells.

[2] Mouse IgG_3 (R_{24}) levels in human serum determined by inhibition enzyme-linked immunoassay.

[3] Human IgG antimouse Ig determined by enzyme-linked immunoassay. Results are presented as the ratio of post-treatment: pre-treatment levels.

NT = not tested.

LT = less than.

ug = microgram